Agricultural Water Management 203 (2018) 344–352

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat

Similarities and differences between the responses to osmotic and ionic stress in quinoa from a water use perspective

Jon Miranda-Apodaca[a], Ander Yoldi-Achalandabaso[a], Aitor Aguirresarobe[a], Arantza del Canto[b], Usue Pérez-López[a,*]

[a] Departamento de Biología Vegetal y Ecología, Facultad de Ciencia y Tecnología, Universidad del País Vasco, UPV/EHU, Apdo. 644, E-48080 Bilbao, Spain
[b] Departamento de Biología Vegetal y Ecología, Facultad de Farmacia, Universidad del País Vasco, UPV/EHU, Pº de la Universidad 7, 01006 Vitoria-Gasteiz, Spain

ARTICLE INFO

Keywords:
Chenopodium quinoa
Drought
Growth
Photosynthesis
Salt stress
Water relations

ABSTRACT

Faced with the consequences of climate change, such as increased drought and salinization of soils, the species *Chenopodium quinoa* may be a good alternative crop because of its high tolerance to these conditions and its high nutritional value. The objective of this work was to analyze the response of the quinoa plant in drought and salinity conditions. Under conditions of drought and severe saline stress (500 mM NaCl), highly similar reductions in growth and relative water content were observed. However, the strategies implemented by the plants in either stress condition and their relative importance were different. Under salt conditions, responses related to osmotic adjustment were more prominent than under drought conditions, where more dehydration was detected. In addition, despite a similar reduction in stomatal conductance in drought and saline conditions, a greater non-stomatal effect was observed in drought conditions, which was demonstrated by the fact that the intercellular CO_2 concentration was increased. Moreover, the antioxidant metabolism also responded differently to the two stresses. Photoassimilate allocation was also different between treatments: the root/shoot ratio remained constant independent of salt concentration, whereas under drought conditions, this ratio increased. A similar trend between treatments was detected for water use efficiency, which was maintained under salt stress and increased under drought conditions, indicating that under reduced water conditions, quinoa can use lower amounts of water per unit of biomass production. These results suggest that *C. quinoa* could be irrigated with brackish or even higher salinity water without severely affecting the growth during its early growth stage, thereby making *C. quinoa* a promising alternative crop for arid and semi-arid regions.

1. Introduction

Climate change is projected to cause a change in the amount of rainfall, increasing in some areas and significantly decreasing in others. Additionally, it is expected to increase extreme events of drought and flooding due to the changes in the distribution of rainfall patterns (IPCC, 2014), and these extreme events are more harmful to ecosystems than changes in the annual mean precipitation (Smith et al., 2005). Furthermore, a rise in temperature is estimated; therefore, higher rates of water evaporation will occur, expanding even more the drought areas. These phenomena, together with agricultural practices, are also causing an increase in the salinization of crop soils (IPCC, 2014). Worldwide, more than 11% of irrigated land are estimated to be salt-affected, a figure that has been rising each year (FAO, 2011a). Water availability is the most important factor in the development of plants (Boyer, 1982; Chaves et al., 2003), therefore under drought- and salt-affected soils, plants suffer water scarcity, and in recent years decreases in crop productivity have been observed.

In addition to the negative impact of climate change causing decreases in productivity, the world population is expected to increase by 52% by the end of the century (UN, 2015), which will require an increase in agricultural production to cope with growing food demand (Tilman et al., 2011). Since 1991, the total agricultural area has remained constant (O'Mara, 2012), so the productivity of crops must increase to meet the growing demand. The increase of drought- and salt affected-soil area, together with the increase in world population, force the research community to find varieties or species that are more tolerant to water limitation or salinity to maintain or increase agricultural production. Recently, efforts have been made in order to improve water use efficiency (*WUE*) of crops to reduce the water needed for agricultural practices (Araus, 2004; Morison et al., 2008; Medrano et al., 2015).

* Corresponding author.
E-mail addresses: jon.miranda@ehu.eus (J. Miranda-Apodaca), ander.yoldi@ehu.eus (A. Yoldi-Achalandabaso), aaguirresarobe001@ikasle.ehu.eus (A. Aguirresarobe), arantza.delcanto@ehu.eus (A. del Canto), usue.perez@ehu.eus (U. Pérez-López).

https://doi.org/10.1016/j.agwat.2018.03.026
Received 29 August 2017; Received in revised form 5 March 2018; Accepted 14 March 2018
Available online 26 March 2018
0378-3774/

Quinoa, *Chenopodium quinoa* Willd, is a halophyte that has been historically cultivated in various regions of South America due to its high adaptability to extreme environments (Bazile et al., 2016). Various authors have observed that quinoa is a highly salt-tolerant crop (Jacobsen et al., 2003; Adolf et al., 2013). Razzaghi et al. (2015) determined that the EC50 for quinoa is 25 dS m^{-1}, and thus confirmed the classification of quinoa as a highly salt-tolerant crop. Additionally, quinoa possesses extraordinary nutritional characteristics, such as high content of protein and essential amino acids (Miranda et al., 2012). All these characteristics led FAO to consider quinoa to be one of the crops that might contribute to global food security during the 21st century (FAO, 2011b).

However, despite the importance of this crop, the studies done till know have analyzed the impact of drought and/or salt stress on the physiological processes separately; for example in water relations (Jensen et al., 2000; Cocozza et al., 2013), in photosynthesis and related parameters (González et al., 2011; Geissler et al., 2015; Razzaghi et al., 2015; Talebnejad and Sepaskhah, 2016), in antioxidant metabolism (Panuccio et al., 2014; Amjad et al., 2015) and in growth (González et al., 2009; Ruiz-Carrasco et al., 2011; Adolf et al., 2012; Ruiz et al., 2016) but never before have been measured all the aforementioned parameters together in just one study. Besides, the studies done comparing the response of quinoa plants to drought and to salt stress are scarce (Bosque-Sanchez et al., 2003; Pulvento et al., 2012; Cocozza et al., 2013; Riccardi et al., 2014; Amjad et al., 2015; Ince Kaya and Yazar, 2016). Thus, the objectives of this study were to determine the different physiological mechanisms that are activated in response to drought and salt stress provoked growth reductions, as well as to compare the response of quinoa to drought and salt stress, highlighting the similarities and differences in the mechanisms that quinoa plants use to cope with the stresses through analysis of various physiological processes as a whole.

2. Materials and methods

2.1. Plant material and experimental design

A Bolivian cultivar of *Chenopodium quinoa* Willd (cv. Real Blanca) was used in this study. Its origin is near the Salar de Uyuni and belongs to *Altiplano* of the Andes ecotype characterized by growing in areas between 3500 and 3900 m above sea level with an annual rainfall of 400–800 mm. Seeds of this cultivar were sown in a 3:1 mixture of perlite/vermiculite in 3 L pots with one seed per pot. Plants were grown from sowing until the end of the experiment in a Conviron PGR15 controlled-environment growth chamber (Conviron, Manitoba, Canada) under a daily 14 h light regimen, an average day/night temperature of 24/20 °C, and a relative day/night humidity of 70/80%. During the light period, the photosynthetic photon flux density (PPFD) was 400 μmol m^{-2} s^{-1}. Light was provided by a combination of incandescent bulbs and warm-white fluorescent lamps. To minimize the effects of intrachamber environmental gradients, plants were randomly repositioned within the chamber each week.

Plants were watered with Hoagland's solution (Arnon and Hoagland, 1940) three times per week maintaining at field capacity until the beginning of the stress treatment, when the plants were 28 days old. Stress treatment was imposed for 14 days. Plants were divided into six groups: five groups were watered every 2 days with 250 mL of Hoagland's solution supplemented with a range of NaCl concentrations (0 mM = control, 60 mM, 120 mM, 240 mM, and 500 mM), as salinity treatments and the sixth group was maintained without watering for 14 days, as drought treatment.

2.2. Measurements of plant water parameters

The leaf relative water content (*RWC*) was measured by gravimetric methods (Pérez-López et al., 2009a). The leaf osmotic potential (Ψ_o) was measured through the freezing point of the cellular sap by an osmometer (Osmomat 030, Gonotec, Germany). The leaf osmotic potential at full turgor (Ψ_o^{100}) was measured following the same procedure as for Ψ_o. To obtain full turgor, leaves were cut from the plants, incubated for 24 h in deionized water, and stored in the dark at 4 °C to avoid the loss of dry mass by respiration or the synthesis of new dry mass by photosynthesis. Dehydration was calculated as the difference between Ψ_o^{100} and the Ψ_o for each treatment. The osmotic adjustment was calculated as the difference between Ψ_o^{100} of the control plants and Ψ_o^{100} of the stressed plants.

The proline concentration was measured following the procedure of Bates et al. (1973). Aliquots of leaf tissue (20 mg lyophilized) were homogenized with 2 mL sulfosalicylic acid at 3%. The homogenates were centrifuged at 16,100*g* for 5 min, and the supernatant was kept on ice. To 0.75 mL of the above supernatant, 0.75 mL of ninhydrin acid was added, consisting of 1.25 g of ninhydrin dissolved in 20 mL of 6 M phosphoric acid and 30 mL of glacial acetic acid. Subsequently, to the mixture of the supernatant and ninhydrin acid, 0.75 mL of glacial acetic acid was added. Samples were incubated for 1 h at 100 °C. Once the tubes had cooled, 1.5 mL of toluene was added, and the mixture was shaken vigorously for 20 s. The fluid separated into two phases, and the upper phase was recovered. Then, the absorbance was determined at 517 nm.

The cumulative plant water transpiration was calculated by gravimetric method. Each pot was weighed every 2 days at the same time, before and after watering (De Luis et al., 1999).

2.3. Gas exchange parameters and chlorophyll a fluorescence

Leaf gas exchange parameters were determined using a Li-6400 open gas exchange system (Li-Cor Inc., Lincoln, NE, USA). Leaf gas exchange rates were measured as described by Pérez-López et al. (2013). Measurements were done 3 h after dawn with a cuvette temperature held at 24 °C and at a relative humidity of 60%. The photosynthetic photon flux density was 400 μmol m^{-2} s^{-1}, provided by red/blue LED light source (model LI 6400-02B, Li-Cor Inc.). The CO_2 concentration of the cuvette (*Ca*) was the same as in growth conditions. Li-Cor software was used to calculate stomatal conductance (*gs*) and the intercellular CO_2 concentration (*Ci*) from net photosynthetic rate (*A*) and instantaneous transpiration rate (*E*) according to the method of von Caemmerer and Farquhar (1981). The intrinsic water use efficiency (*A*/*gs*) was calculated by dividing *A* by *gs*.

The actual photochemical efficiency of photosystem II ($\Phi PSII = (Fm' - Fs)/Fm'$) was determined by measuring steady-state fluorescence (*Fs*) and maximum fluorescence during a light-saturating pulse of ~8000 μmol m^{-2} s^{-1} (*Fm'*) following the procedures of Genty et al. (1989). The electron transport rate (*ETR*) indicates the proportion of light used in photochemical processes of the total of energy that reaches the leaf per unit of time and surface (PPFD), and it was calculated according to the following formula: $ETR = \Phi PSII x PPFD x 0.85 \times 0.5$. The absorption coefficient of the leaves was taken as 0.85 and the fraction of the excitation energy distributed to PSII was taken as 0.5 (Genty et al., 1989).

Chlorophylls and carotenoids were extracted in dimethylsulfoxide (DMSO) as described by Barnes et al. (1992). The absorbance was measured at 665, 649, and 480 nm to quantify the pigment concentration using the formulas proposed by Wellburn (1994).

2.4. Antioxidant enzyme activities

All operations were carried out at 0–4 °C. Aliquots of leaf tissue (0.15 g fresh weight) were ground in a cold mortar using extraction buffer (3 mL). For catalase (CAT) activity, the extraction buffer consisted of 50 mM Tris–HCl (pH 7.8), 0.1 mM ethylenediaminetetraacetate (EDTA), 0.2% Triton X-100 (v/v), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM dithiotreitol (DTT). The

homogenates were squeezed through two layers of muslin and centrifuged at 16,100*g* for 25 min. CAT activity was measured spectrophotometrically according to the method of Pérez-López et al. (2009b) by monitoring the disappearance of H_2O_2 at 240 nm for 6 min at 25 °C. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7) and 20 mM H_2O_2, and 5 μL of the supernatant was added to start the reaction. Until quantification, H_2O_2 was kept in the dark.

For ascorbate peroxidase (APX) activity, the extraction buffer consisted of 50 mM potassium phosphate buffer (pH 7.8) containing 2% polyvinylpolypyrrolidone (PVPP; w/v), 0.1 mM EDTA, 0.1 mM PMSF, 0.2% Triton X-100 (v/v), 5 mM cysteine, and 2 mM ascorbate. Ascorbate was added to the medium to avoid inactivation of APX during the extraction and assay. The homogenates were squeezed through two layers of muslin and centrifuged at 16,100*g* for 25 min. APX activity was assayed by measuring the oxidation of ascorbate at 290 nm according to the method of Pérez-López et al. (2010). The reaction mixture contained 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes, pH 6.6) and 0.8 mM ascorbate. The oxidation rate of ascorbate, measured as the decline in absorbance at 290 nm, was estimated between 1 and 60 s after starting the reaction with the addition of 0.8 mM H_2O_2 (10 μL) and 10 μL of the supernatant. Corrections were made for the non-enzymatic oxidation of ascorbate by H_2O_2 and for the oxidation of ascorbate in the absence of H_2O_2.

The protein content was measured according to the method of Bradford (1976), using bovine serum albumin as a standard.

2.5. Growth parameters

At the end of the experiment (day 42), the plants were harvested, separated into leaves, stems, and roots, and weighed to determine fresh weight (FW). After that, the samples were dried at 80 °C for 48 h, and their dry weight (DW) was determined.

Whole plant water use efficiency (*WUE*) was calculated as the estimated dry weight accumulation per plant divided by the cumulative transpiration per plant (Pérez-López et al., 2014).

2.6. Statistical analysis

Results are reported as the mean ± standard error (SE) of four independent replicates. The SE was directly calculated from crude data. Data analyses were performed using the SPSS 19.0 software package (Chicago, IL). One-way analysis of variance (ANOVA) was used to evaluate the main effects of the stress. Means were compared using Duncan's multiple range test. *P*-values ≤ 0.05 were considered statistically significant. Prior to analyses, we tested whether the assumptions of an ANOVA, homogeneity of variances and normally distributed residuals were achieved. The homogeneity of variances for all the studied parameters was evaluated by Levene's test and the distribution of the residuals was assessed by Kolmogorov–Smirnov test. When necessary, transformation was applied before statistical analysis was performed. Principal component analysis (PCA) was performed using R (R Core Team, 2016), all treatments and variables were included in the analysis.

3. Results

3.1. Leaf water status

Intermediate saline concentrations (120 and 240 mM) resulted in 9% lower leaf relative water content (*RWC*), whereas the highest salt concentration (500 mM) and drought reduced *RWC* by 17% (Fig. 1A) compared to control values (well-watered with 0 mM NaCl). The leaf osmotic potential (Ψ_o) was more negative with higher saline concentrations in the irrigation water (Fig. 1B). High salinity (500 mM) and drought caused a statistically similar reduction in this parameter, with values close to -2 MPa.

The decrease in Ψ_o was due both to dehydration (passive mechanism) and osmotic adjustment (active mechanism). However, the contribution of each mechanism to the decrease in Ψ_o was different depending on the treatment (Table 1). Plants subjected to drought treatment showed more dehydration, reaching values of 0.70 MPa, than plants exposed to saline treatment, where only in the 500 mM treatment dehydration was detected. In contrast, in the plants subjected to saline treatment, a greater capacity for osmotic adjustment was observed, up to 0.97 MPa in the 500 mM treatment.

Together with the increase in the osmotic adjustment capacity, the proline concentration increased (Table 1). In fact, there was a close correlation between proline concentration and osmotic adjustment among the treatments ($r^2 = 0.86$; $P < 0.01$). The highest level of proline, 10.8 nmol g^{-1} DW, was observed in the most severe saline treatment (500 mM). Proline levels were 40% lower in plants subjected to 240 mM NaCl, as well as in plants subjected to drought compared to control values. Treatments of 60 and 120 mM NaCl did not affect the concentration of this amino acid compared to the control conditions.

3.2. Gas exchange and related parameters

As long as saline treatment became more severe, the net photosynthetic rate (*A*) tended to decrease, although the observed differences were not statistically significant at concentrations lower than 240 mM NaCl (Fig. 2A). Nevertheless, at the highest salt concentration (500 mM), *A* was 65% lower than in controls. On the other hand, drought treatment resulted in 77% lower values for *A*. The stomatal conductance (*gs*) decreased along the salinity gradient, with the largest reduction of 83% seen with 500 mM NaCl (Fig. 2B). The effect of drought treatment had the same magnitude as the highest saline concentration. The salt treatments affected the intercellular CO_2 concentration (*Ci*): it tended to decrease with more severe stress (Fig. 2C). On the other hand, drought treatment resulted in a 25% increase in *Ci*. The carboxylation capacity (expressed as *A/Ci*) was 38% lower with 500 mM NaCl, and the reduction was even greater under drought conditions, which resulted in a decline of 79% (Fig. 2D). Because *gs* decreased proportionally more than *A* did, the intrinsic water use efficiency (*A/gs*) increased in all saline conditions, with the highest value being at 500 mM, where an increase of 100% was detected (Fig. 2E). The effect of drought on *A/gs* was similar to the 240 mM saline treatment, with an increase of 60%. Instantaneous transpiration rate (*E*) followed a similar pattern to *gs*: it decreased with the salinity gradient, with a maximum decrease of 77% at 500 mM (Fig. 2F) compared to control values. The effect of the drought treatment was similar to the 500 mM saline treatment, showing a decrease of 83% with respect to control values.

The electron transport rate (*ETR*) did not change with salt concentration. However, the drought treatment reduced *ETR* by 58% compared to control values (Fig. 3A). The concentration of chlorophyll *a* (Chl-*a*) was not altered in any saline treatment. Additionally, drought treatment caused a slight increase in Chl-*a* concentration, although it was not statistically significant (Fig. 3B). As with Chl-*a*, the concentration of chlorophyll *b* (Chl-*b*) was not altered by any saline treatment, although at the highest salt concentration, Chl-*b* was slightly higher, although this increase was not statistically significant (Fig. 3C). Drought caused a significant increase in Chl-*b,* up to 60% compared to control values. The increases in Chl-*b* under drought treatment caused a decrease in the Chl *a/b* ratio, which was 22% compared to control values. The effect of saline treatments and drought on carotenoid concentration was similar to Chl-*b*, following the same pattern (Fig. 3D), that is, an increase with drought treatment (19%) was detected.

The ascorbate peroxidase (APX) activity was only altered at the highest salt concentration, 58% higher than control values (Fig. 4A). At the same time, drought resulted in increased APX activity (43%) similar to the values detected with the 500 mM salt treatment. The salt treatments resulted in higher catalase (CAT) activity, which was 81% higher

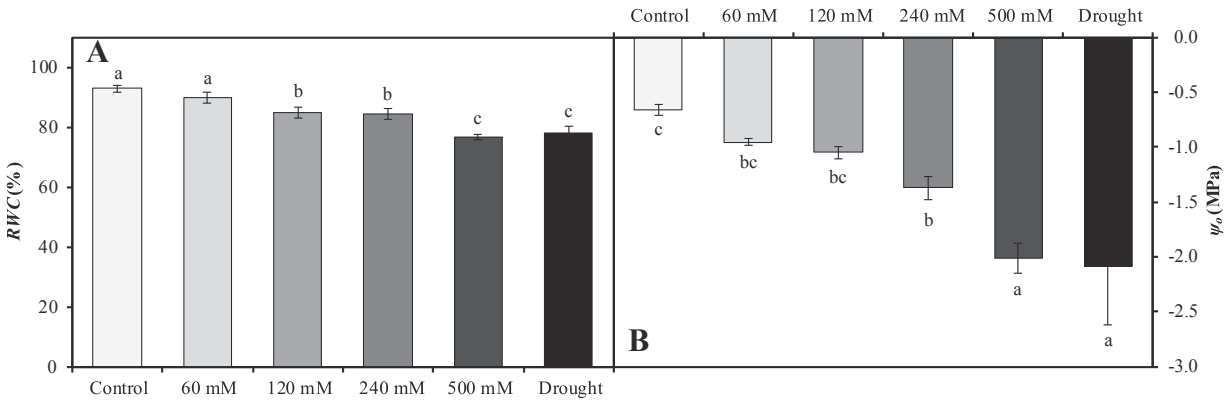

Fig. 1. Leaf relative water content (*RWC*, A) and osmotic potential (Ψ_o, B) in *Chenopodium quinoa* plants subjected to control (white bars), different salt concentrations (ranging from 60 mM to 500 mM NaCl, colored in gray-scale bars) and drought (black bars) conditions. Values represent mean ± SE values of 4 plants per treatment. Different letters indicate significant differences ($P \leq 0.05$) between treatments.

Table 1
Effects of drought and salt stress on osmotic adjustment (*OA*; MPa), dehydration (*DH*; MPa), and proline concentration (nmol g^{-1} DW).

Treatments	*OA*	*DH*	Proline
Control		−0.11 ± 0.02 c	3.62 ± 0.93 c
60 mM	0.14 ± 0.06 c	0.05 ± 0.10 bc	3.90 ± 0.40 c
120 mM	0.41 ± 0.05 ab	−0.12 ± 0.05 c	4.24 ± 0.48 c
240 mM	0.48 ± 0.10 ab	0.13 ± 0.10 bc	7.48 ± 0.49 b
500 mM	0.97 ± 0.08 a	0.28 ± 0.12 b	10.79 ± 1.51 a
Drought	0.35 ± 0.28 bc	0.70 ± 0.24 a	6.55 ± 0.58 b

with 500 mM NaCl than control (Fig. 4B). Drought treatment resulted in similar values as the control and 60 mM NaCl, the lowest salt treatment measured in this experiment.

3.3. Biomass production, cumulative transpiration and water use efficiency

Total biomass (roots, stems and leaves) decreased with salt concentrations above 120 mM. The effects of drought and the highest salt concentration (500 mM) on biomass were similar, showing a 44–48% reduction of the total dry weight compared to the control values. Analyzing organ by organ, only the highest salinity concentrations (240 and 500 mM) caused a negative effect on leaf biomass (Fig. 5A). On the other hand, the effect of drought was statistically comparable to the values obtained with concentrations of 500 mM, reducing leaf biomass by 50%. Stem biomass was negatively affected both by high saline concentrations and drought, showing declines of 43% (500 mM NaCl) and 53% (drought) compared to control values. In addition, it was observed that plants subjected to a 240 mM saline stress had 28% less biomass compared to the previous lower concentrations. Finally, the various concentrations of salinity had a similar effect on the root dry weight biomass as was detected for shoot biomass, so the root/shoot ratio remained constant. In drought conditions, the reduction in root biomass was 26% compared to control values; thus the decrease in shoot biomass was greater than the decrease in root biomass, so the root/shoot biomass ratio increased comparing to control values.

The cumulative plant water transpiration decreased gradually with increases in salinity gradient, reaching a final reduction of 42% at 500 mM (Fig. 5B) compared to control values. The effect of the drought treatment was more severe, resulting in a decrease of 63% compared to control. Finally, when we calculated the water use efficiency (*WUE*) of the plants, we saw no differences in this parameter with various saline concentrations, except for 120 mM NaCl, where an increase of 15% occurred (Fig. 5C). In contrast, drought treatment increased *WUE* by 55% compared to control values.

3.4. Principal component analysis

Principal component analysis (PCA) analysis was performed to display the maximum amount of variation in a data profile within a few principal components and to understand relations between variables. The plots depict standardized scores along the first two components. The first two components explain 85% of the data variability (Fig. 6). The PC1 (62% of data variability) separated treatment according to their severity, one group consisted of control, 60, and 120 mM NaCl treatments and the other group of drought and 500 mM NaCl treatments. The variables most correlated positively with PC1 were some stress tolerance mechanisms such as osmotic adjustment, proline concentration, and APX activity. On the other hand, water relations, photosynthetic processes and biomass production correlated negatively with PC1. The PC2 (23% of data variability) divided high salinity treatments and drought and it contained in one side stress tolerance mechanisms such as CAT activity, osmotic adjustment, and *A/gs* ratio and in the other side *Ci*.

4. Discussion

4.1. Leaf water status

Water availability is an important factor that can compromise leaf water status. In the present study, drought and salt stress reduced quinoa leaf *RWC* (Fig. 1A). Jensen et al. (2000) for drought and Cocozza et al. (2013) for different drought and salt stress treatments also found decreases in *RWC*. In fact, Cocozza et al. (2013) detected a close relationship between *RWC* and Ψ_o. We also detected decreases in Ψ_o. Besides, Ψ_o decreased to similar values under 500 mM NaCl and drought conditions (Fig. 1B). However, the mechanisms that explain the reduction in Ψ_o were different, the response being dependent on the treatment. Under saline conditions, the reduction in Ψ_o was gradual and dependent on dehydration (passive mechanism) and osmotic adjustment (active mechanism). Shabala et al. (2012) and Cocozza et al. (2013) also measured osmotic adjustment capacity in quinoa. However, the relative contribution of both processes has never been measured before in quinoa. With more severe saline treatment, the importance of osmotic adjustment compared to dehydration increased. A similar combination of these mechanisms acting together has been detected in other species, such as barley (Pérez-López et al., 2009a). When we analyzed the response of Ψ_o in quinoa to drought, we observed that even if the plant could undergo osmotic adjustment, dehydration was more important than osmotic adjustment for the same value of leaf *RWC*. Dehydration usually implies loss of turgor, so for the same *RWC* salt-treated plants tolerate water stress better than drought-treated plants.

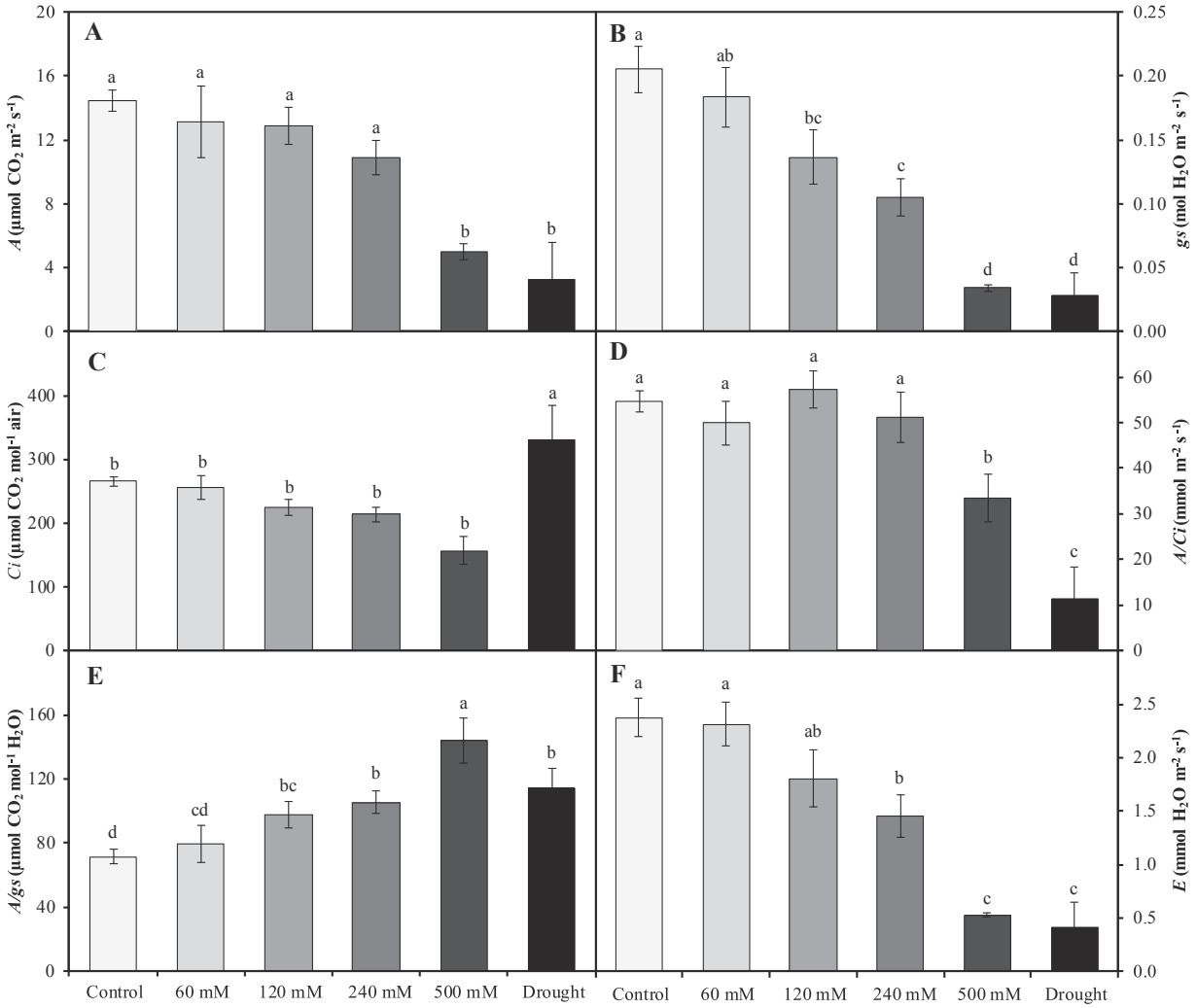

Fig. 2. Net photosynthetic rate (*A*, A), stomatal conductance (*gs*, B), intercellular CO_2 concentration (*Ci*, C), carboxylation capacity (*A/Ci*, D), intrinsic water use efficiency (*A/gs*, E) and instantaneous transpiration rate (*E*, F). Growth conditions and statistical analysis are as described in Fig. 1.

As mentioned above, although it occurred in different proportions, under both stresses quinoa plants underwent osmotic adjustment. It has been shown that 85% of osmotic adjustment in quinoa leaves is achieved by means of the accumulation of inorganic ions (Hariadi et al., 2011). However, the use of compatible solutes is necessary for osmotic adjustment in the cytosol (Adolf et al., 2013). Different components, such as proline, glycinebetaine, polyamines, and others, have been ascribed to function as compatible solutes under drought and saline conditions (Flowers and Colmer, 2008; Shabala et al., 2012). We detected a strong correlation between osmotic adjustment and proline independently of the treatment, also confirmed by PCA analysis (Fig. 6), so in this paper we focused on the relevance of proline in quinoa.

An increase in proline has been proposed as an adaptive response to stress (Singh et al., 2000; Lin et al., 2002). However, in this respect there is still much debate, as some authors have associated it with a mechanism of tolerance to salinity, and others, in contrast, to a negative consequence of salinity. Madan et al. (1995) and Silveira et al. (2001) found that salinity-tolerant cultivars had higher proline content than sensitive ones, whereas Lutts et al. (1999) and Lacerda et al. (2003) reported higher concentrations of proline in sensitive cultivars. In our case, the proline levels were positively correlated with osmotic adjustment ($r^2 = 0.86$; $P < 0.01$). These results would point to proline as a marker for tolerance because the treatments with a higher content of this amino acid were associated with higher osmotic adjustment capacity. Reinforcing this idea, Ruiz-Carrasco et al. (2011) found higher proline concentration in quinoa accessions that were most salt-tolerant than in the ones that were less salt-tolerant.

4.2. Gas exchange and related parameters

The availability of CO_2 is another important factor that can compromise biomass production. As it is seen in Fig. 2B, the *gs* tended to decrease with more severe saline treatment, and we detected similar values for *gs* at 500 mM NaCl and drought treatments. The reductions in *gs* have been ascribed to limited water uptake and decreases in leaf water content (Adolf et al., 2012, 2013; Amjad et al., 2015). The fact that the values for *gs* (Fig. 2B) and *RWC* (Fig. 1A) were similar in 500 mM NaCl and drought conditions support this idea. Together with decreases in *gs*, we detected decreases in *A* (Fig. 2A), as also other authors did (Jacobsen et al., 2009; González et al., 2011; Geissler et al., 2015; Razzaghi et al., 2015; Talebnejad and Sepaskhah, 2016). Several authors have described that when the water stress is mild the reduction in photosynthetic rate is due to stomatal limitations (Lawlor and Tezara, 2009; Chaves et al., 2009). At concentrations lower than 240 mM NaCl, the reduced *gs* limited the uptake and diffusion of CO_2 to the carboxylation site, as reflected by the decline in *Ci* (Fig. 2C) and *Ci/Ca* ratio. The maintenance of the carboxylation capacity (expressed as *A/Ci*; Fig. 2D) supports the idea that until 240 mM NaCl *A* is mostly limited by stomatal conductance. However, with 500 mM NaCl and drought treatments, the *A/Ci* decreased significantly, indicating that

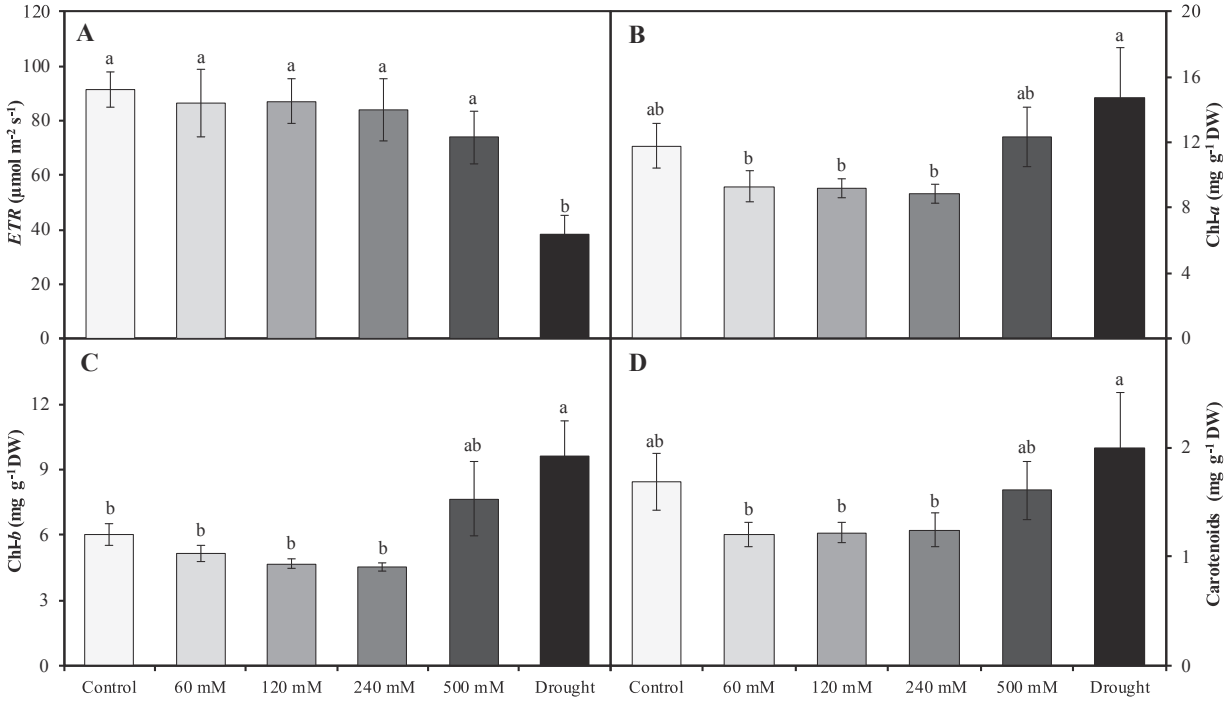

Fig. 3. Electron transport rate (*ETR*, A), chlorophyll-*a* (Chl-*a*, B), chlorophyll-*b* (Chl-*b*, C) and carotenoid concentration (D). Growth conditions and statistical analysis are as described in Fig. 1.

limitations other than stomatal could also have occurred in these treatments. Razzaghi et al. (2015) also found that under drought photosynthesis was affected via *gs* and photosynthetic limitations. Additionally, the decreases were more marked in drought conditions than in 500 mM NaCl conditions. The lower *A/Ci* in drought conditions was mainly caused by an increase in *Ci* (Fig. 2C), indicating high non-stomatal limitations. These limitations could depend on reductions in chlorophyll concentration and/or inadequate functioning of the photosystems (photochemical limitations), and/or reductions in the activity or synthesis of Calvin cycle enzymes (biochemical limitations) (Lawlor and Tezara, 2009; Chaves et al., 2009).

To examine possible photochemical limitations, we studied *ETR* (Fig. 3A) and the concentration of chlorophylls and carotenoids (Fig. 3B–D). The *ETR* only decreased under drought treatment, which could explain the lower *A/Ci* values under drought conditions compared to 500 mM NaCl conditions. Regarding pigments, Geissler et al. (2015) stated that a reduction in chlorophyll concentration can cause a reduction in photosynthesis and, thus, explain part of the non-stomatal limitations. However, based on the results of pigment analysis, which tended to increase under 500 mM NaCl and drought (Fig. 3B–D), we assume that the decrease in photosynthesis was not caused by a lower pigment concentration. Generally, drought and high saline treatments cause reductions in photosynthetic pigments (González et al., 2011; Amjad et al., 2015; Ruiz et al., 2016), usually because their synthesis is inhibited or because the chlorophyllase activity is high (Majumdar et al., 1991; Loggini et al., 1999). However, we found the opposite trend when the saline or drought stress was severe. These contradictory results could indicate that in our cultivar, the increases in chlorophyll concentration detected under severe stress conditions could be due to a concentrating effect of chlorophylls because the biomass reduction was proportionally greater than the increase in chlorophyll degradation. The increase in chlorophylls due to concentration effect under stress was also argued by Hajiboland et al. (2011) in tea plants.

Continuing with the causes of the decrease in biomass production, we need to consider the oxidative stress caused by saline and drought treatments. Whenever a decrease in net photosynthetic rate occurs, an imbalance between photochemical and biochemical phases of the photosynthesis happens. This occurs because the electron transport rate and consequent ATP and NADPH production exceeds its demand in the Calvin cycle if the energy absorption does not decrease, e.g., through a lower concentration of pigments, which was not observed under 500 mM NaCl and drought conditions. In fact, we detected a higher

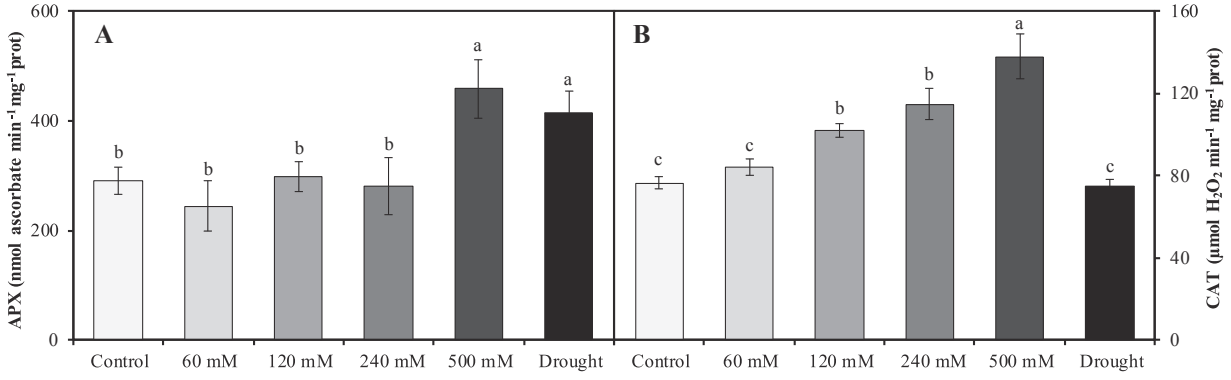

Fig. 4. Ascorbate peroxidase (APX, A) and catalase (CAT, B) activities. Growth conditions and statistical analysis are as described in Fig. 1.

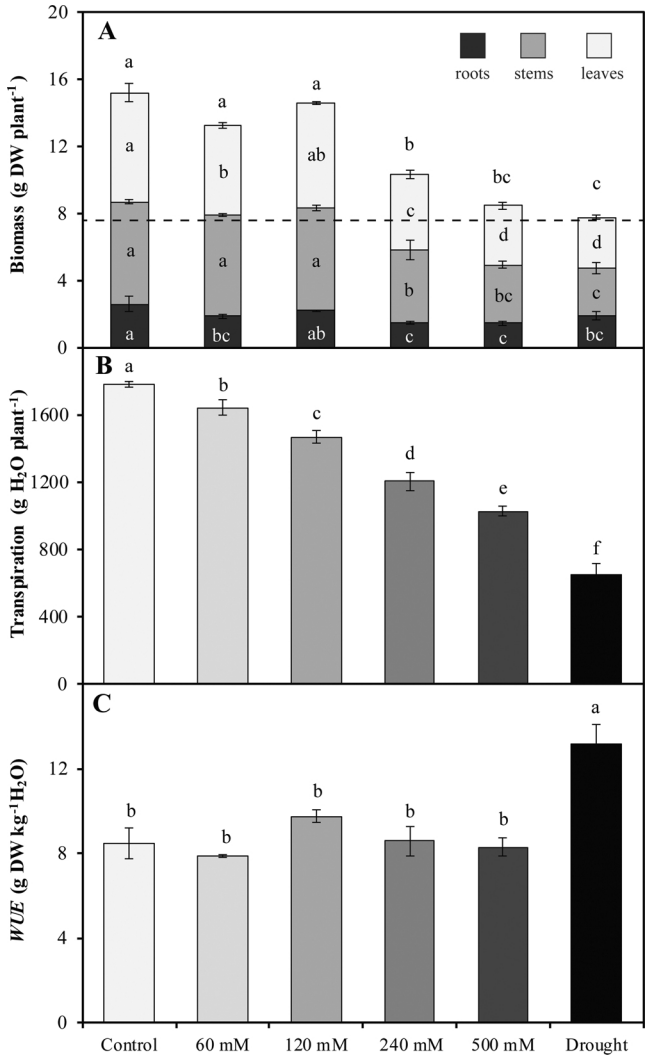

Fig. 5. Total, leaf, stem and root dry biomass (A), cumulative transpiration (B) and water use efficiency (*WUE*, C). Growth conditions and colors for B and C are as described in Fig. 1. Statistical analysis is as described in Fig. 1. The dashed line marks the reduction of the total dry biomass by 50% (P_{50} value).

ETR/A ratio, which corresponds to a higher risk of reactive oxygen species (ROS) production. These ROS can cause oxidative stress if they are not properly removed. Ascorbate peroxidase (APX) and catalase (CAT) enzymes are important for scavenging these ROS, such as H_2O_2.

We detected increases in APX activity with 500 mM NaCl and drought treatments (Fig. 4A). It seems that with concentrations of 240 mM NaCl or lower, oxidative stress did not occur, as *A* did not decrease, but once the threshold value of 240 mM NaCl was passed, the effects of oxidative burst came into play, as evidenced by the decrease in *A*. Increases in APX activity to reduce oxidative stress have also been detected in quinoa plants subjected to drought (600 mM mannitol) and saline (200–400 mM NaCl) treatments (Panuccio et al., 2014; Amjad et al., 2015). In the PCA analysis, APX clustered together with osmotic adjustment and proline (Fig. 6) indicating that is functioning as a tolerance mechanism, as osmotic adjustment did.

Catalase is another enzyme that it is important for scavenging H_2O_2 produced in the peroxisome due to the activity of photorespiration. With more severe saline treatment, the CAT activity increased progressively (Fig. 4B), indicating that Rubisco carboxylation activity was reduced while the oxygenation activity was increased. Several authors have also observed increases in CAT due to saline and drought treatments (Panuccio et al., 2014; Amjad et al., 2015). Regarding drought treatment, because *A* was reduced, we also expected to detect increases in CAT activity. However, we detected similar values as control conditions. This result implies that under drought conditions, the lower *ETR* decreased the quantity of electrons directed to photorespiration and CO_2 assimilation reactions, considered as a whole. Since under drought conditions the proportion of energy used in photorespiration is presumably higher than in the control plants, the absolute value of photorespiration could be similar to the one detected than in control plants (even if *ETR* is lower). Our results regarding CAT activity maintenance seem to support this hypothesis.

4.3. Biomass production, cumulative transpiration and water use efficiency

Quinoa is a highly salt-tolerant plant. Razzaghi et al. (2015) determined the EC50 for quinoa to be 25 dS m^{-1}, although differences between cultivars have been detected (Ruiz-Carrasco et al., 2011; Adolf et al., 2012; Shabala et al., 2012; Ruiz et al., 2016). For our cultivar, Real Blanca, when saline stress was moderate (60–120 mM NaCl), *RWC* and *A* values were similar to the control treatment, and therefore biomass production was maintained. However, at 240 mM NaCl and higher concentrations, reductions in *RWC* and *A* started to occur, which ultimately caused biomass reductions (Fig. 5A). In fact, a greater decrease in *RWC* and *A* was related to a greater biomass reduction. This is corroborated by the analysis of PCA (Fig. 6) where it seems that *A*, *RWC*, and total biomass were clustered together, indicating correlation between these variables. The decrease of biomass with saline conditions was proportional among organs, so the root/shoot ratio was kept constant, independent of the salt concentration. Ruiz-Carrasco et al. (2011) carried out an experiment with 4*C. quinoa* genotypes and detected increases, decreases and maintenance of the root/shoot ratio, indicating that the response of root/shoot ratio is dependent on the genotype. Our results would demonstrate that under saline conditions, in Real Blanca cv. all the organs are important for surviving and tolerating salt stress, so there is a need to allocate carbohydrates, on one hand, to the roots to continue the uptake of water and, on the other hand, to the shoots to maintain photosynthetic capacity and to accumulate salt into salt bladders. As occurred in the high salinity treatments, under drought conditions, decreases in *RWC* and *A* were detected and we observed biomass reduction. Conversely to what happened under salinity, this decrease was not uniform among the organs. The root contribution increased compared to control conditions, thus increasing the root/shoot ratio. This would indicate, following the functional equilibrium theory (Brouwer, 1962), that the root is the organ responsible for the uptake of the limiting nutrient, in this case, water, and that Real Blanca cv. allocates more photoassimilates to roots in order to try to increase water uptake.

Moreover, the decrease in biomass both under saline and drought conditions may be a positive characteristic, because it reduces transpiration from the leaf surface and thus avoids excessive loss of water (Sun et al., 2014) or excessive uptake of Na and Cl (Melgar et al., 2008). We observed a reduction in the cumulative transpiration (Fig. 5B). This reduction was higher than the reduction in biomass in drought conditions (Fig. 5A), thus we detected an increase in *WUE* (Fig. 5C) in this condition, indicating that under reduced water conditions, quinoa can use lower amounts of water per unit of biomass production. Under salt stress, the *WUE* had similar values compared to controls independently of NaCl stress severity. This result indicates that quinoa did not reduce water use, but still maintained biomass production up to 120 mM NaCl. In the majority of the studies done in quinoa, the salt or drought stress was imposed in flowering stage (Jensen et al., 2000; Bosque-Sanchez et al., 2003; Razzaghi et al., 2012a,b; Fischer et al., 2013; Riccardi et al., 2014; Geissler et al., 2015; Bascuñán-Godoy et al., 2016). However, in this study, the stress was applied at vegetative stage and we still detected high ability to tolerate salt and drought stress. This has important economic implications because it means that quinoa is also salt-tolerant during the early stages of development, which would permit farmers to water quinoa in the early stages of development with

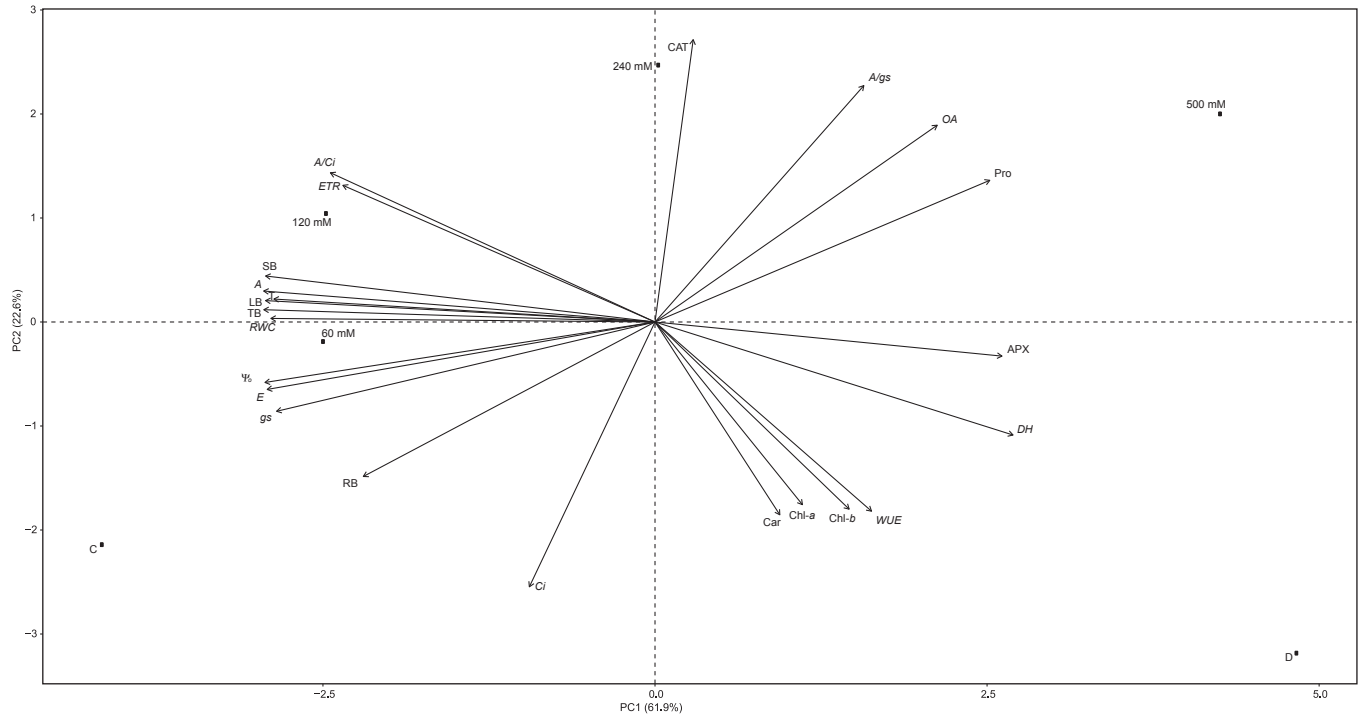

Fig. 6. Principal component analysis (PCA). Biplot based on PC1 and PC2. Percentages correspond to how much data variability is explained by the respective component. Projections of the arrows on the axes indicate with which variables the PCs are linked the most. The arrow closeness indicates how much correlation exists between the variables. For treatments: C, control; D, drought; 60 mM, 60 mM NaCl salinity; 120 mM, 120 mM NaCl salinity; 240 mM, 240 mM NaCl salinity; 500 mM, 500 mM NaCl salinity. For variables, *A*, net photosynthetic rate; APX, ascorbate peroxidase activity; *A/Ci*, carboxylation capacity; *A/gs*, intrinsic water use efficiency; Car, carotenoids; CAT, catalase activity; Chl-*a*, chlorophyll *a*; Chl-*b*, chlorophyll *b*; *Ci*, intercellular CO_2 concentration; *DH*, dehydration; *E*, instantaneous transpiration rate; *ETR*, electron transport rate; *gs*, stomatal conductance; LB, leaf biomass; *OA*, osmotic adjustment; Pro, proline; RB, root biomass; *RWC*, leaf relative water content; SB, stem biomass; TB, total biomass; T, transpiration; *WUE*, water use efficiency; Ψ_o, leaf osmotic potential.

brackish water or even with high-salinity water (Ince Kaya and Yazar, 2016).

In conclusion, the highest salinity and drought treatments provoked similar growth responses, but the mechanisms activated were different, corroborated by PCA analysis where drought and 500 mM NaCl clustered in opposite sides of the PC2. Under salinity, the osmotic adjustment capacity was higher and the photosynthetic non-stomatal limitations lower than under drought conditions. The antioxidant metabolism also responded differently to the two stresses. Moreover, under saline conditions the root/shoot ratio was maintained, whereas under drought conditions this ratio increased. Therefore, photoassimilate allocation patterns differed between treatments. Besides, in the lowest salt concentrations (60–120 mM NaCl) biomass production did not decrease, which would permit farmers to water quinoa in the early stages of development with brackish water or even water with higher salinity. Under drought conditions, the water use efficiency increased, indicating that quinoa can be grown in water-limited areas. These findings emphasize the usefulness of Real Blanca as a promising cultivar that could be grown in water-limited areas or on saline soils, which are expected to increase with future environmental conditions.

Acknowledgements

This research was financially supported by the following grants: University of the Basque Country (EHUA16/17), UFI11/24, and GRUPO Gobierno Vasco-IT1022-16. We also thank IZAITE, Ehne and Factor CO_2 for their support.

References

Adolf, V.I., Shabala, S., Andersen, M.N., Razzaghi, F., Jacobsen, S.-E., 2012. Varietal differences of quinoa's tolerance to saline conditions. Plant Soil 357, 117–129.
Adolf, V.I., Jacobsen, S.-E., Shabala, S., 2013. Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). Environ. Exp. Bot. 92, 43–54.
Amjad, M., Akhtar, S.S., Yang, A., Akhtar, J., Jacobsen, S.E., 2015. Antioxidative response of quinoa exposed to iso-osmotic, ionic and non-ionic salt stress. J. Agron. Crop Sci. 201, 452–460.
Araus, J., 2004. The problems of sustainable water use in the Mediterranean and research requirements for agriculture. Ann. Appl. Biol. 144, 259–272.
Arnon, D.I., Hoagland, D.R., 1940. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. 50, 463–485.
Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W., 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. Environ. Exp. Bot. 32, 85–100.
Bascuñán-Godoy, L., Reguera, M., Abdel-Tawab, Y.M., Blumwald, E., 2016. Water deficit stress-induced changes in carbon and nitrogen partitioning in *Chenopodium quinoa* Willd. Planta 243, 591–603.
Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.
Bazile, D., Pulvento, C., Verniau, A., Al-Nusairi, M.S., Ba, D., Breidy, J., Hassan, L., Mohammed, M.I., Mambetov, O., Otambekova, M., Sepahvand, N.A., Shams, A., Souici, D., Miri, K., Padulosi, S., 2016. Worldwide evaluations of quinoa: preliminary results from post international year of quinoa FAO projects in nine countries. Front. Plant Sci. 7.
Bosque-Sanchez, H., Lemeur, R., Van Damme, P., Jacobsen, S.E., 2003. Ecophysiological analysis of drought and salinity stress of quinoa (*Chenopodium Quinoa* Willd.). Food Rev. Int. 19, 111–119.
Boyer, J.S., 1982. Plant productivity and environment. Science 218, 443–448.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Brouwer, R., 1962. Distribution of dry matter in the plant. Neth. J. Agric. Sci. 10, 361–376.
Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003. Understanding plant responses to drought-from genes to the whole plant. Funct. Plant Biol. 30, 239–264.
Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560.
Cocozza, C., Pulvento, C., Lavini, A., Riccardi, M., d'Andria, R., Tognetti, R., 2013. Effects of increasing salinity stress and decreasing water availability on ecophysiological traits of quinoa (*Chenopodium quinoa* Willd.) grown in a mediterranean-type agroecosystem. J. Agron. Crop Sci. 199, 229–240.
De Luis, I., Irigoyen, J.J., Sánchez-Díaz, M., 1999. Elevated CO_2 enhances plant growth in droughted N_2-fixing alfalfa without improving water status. Physiol. Plant. 107, 84–89.
FAO, 2011a. The State of the World's Land and Water Resources for Food and Agriculture (SOLAW) – Managing Systems at Risk. Food and Agriculture Organization of the

United Nations, Rome and Earthscan, London.
FAO, 2011b. Quinoa: An Ancient Crop to Contribute to World Food Security.
Fischer, S., Wilckens, R., Jara, J., Aranda, M., 2013. Variation in antioxidant capacity of quinoa (*Chenopodium quinoa* Will) subjected to drought stress. Ind. Crop. Prod. 46, 341–349.
Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963.
Geissler, N., Hussin, S., El-Far, M.M.M., Koyro, H.-W., 2015. Elevated atmospheric CO_2 concentration leads to different salt resistance mechanisms in a C_3 (*Chenopodium quinoa*) and a C_4 (*Atriplex nummularia*) halophyte. Environ. Exp. Bot. 118, 67–77.
Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.
González, J.A., Gallardo, M., Hilal, M., Rosa, M., Prado, F.E., 2009. Physiological responses of quinoa (*Chenopodium quinoa* Willd.) to drought and waterlogging stresses: dry matter partitioning. Bot. Stud. 50, 35–42.
González, J.A., Bruno, M., Valoy, M., Prado, F.E., 2011. Genotypic variation of gas exchange parameters and leaf stable carbon and nitrogen isotopes in ten quinoa cultivars grown under drought. J. Agron. Crop Sci. 197, 81–93.
Hajiboland, R., Bastani, S., Bahrami-Rad, S., 2011. Effect of light intensity on photosynthesis and antioxidant defense in boron deficient tea plants. Acta Biol. Szeged. 55, 265–272.
Hariadi, Y., Marandon, K., Tian, Y., Jacobsen, S.-E., Shabala, S., 2011. Ionic and osmotic relations in quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. J. Exp. Bot. 62, 185–193.
IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (Geneva, Switzerland).
Ince Kaya, Ç., Yazar, A., 2016. Saltmed model performance for quinoa irrigated with fresh and saline water in a mediterranean environment. Irrig. Drain. 65, 29–37.
Jacobsen, S.E., Mujica, A., Jensen, C.R., 2003. The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. Food Rev. Int. 19, 99–109.
Jacobsen, S.E., Liu, F., Jensen, C.R., 2009. Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd.). Sci. Hortic. 122, 281–287.
Jensen, C.R., Jacobsen, S.E., Andersen, M.N., Núñez, N., Andersen, S.D., Rasmussen, L., Mogensen, V.O., 2000. Leaf gas exchange and water relation characteristics of field quinoa (*Chenopodium quinoa* Willd.) during soil drying. Eur. J. Agron. 13, 11–25.
Lacerda, C.F., Cambraia, J., Oliva, M.A., Ruiz, H.A., Prisco, J.T., 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ. Exp. Bot. 49, 107–120.
Lawlor, D.W., Tezara, W., 2009. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Ann. Bot. 103, 561–579.
Lin, C.C., Hsu, Y.T., Kao, C.H., 2002. The effect of NaCl on proline accumulation in rice leaves. Plant Growth Regul. 36, 275–285.
Loggini, B., Scartazza, A., Brugnoli, E., Navari-Izzo, F., 1999. Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol. 119, 1091–1100.
Lutts, S., Majerus, V., Kinet, J.M., 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol. Plant. 105, 450–458.
Madan, S., Nainawatee, H.S., Jain, R.K., Chowdhury, J.B., 1995. Proline and proline metabolising enzymes in in-vitro selected NaCl-tolerant *Brassica juncea* L. under salt stress. Ann. Bot. 76, 51–57.
Majumdar, S., Ghosh, S., Glick, B.R., Dumbroff, E.B., 1991. Activities of chlorophyllase, phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase in the primary leaves of soybean during senescence and drought. Physiol. Plant. 81, 473–480.
Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.-M., Bota, J., 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop J. 3, 220–228.
Melgar, J.C., Syvertsen, J.P., García-Sánchez, F., 2008. Can elevated CO_2 improve salt tolerance in olive trees? J. Plant Physiol. 165, 631–640.
Miranda, M., Vega-Gálvez, A., Quispe-Fuentes, I., Rodríguez, M.J., Maureira, H., Martínez, E.A., 2012. Nutritional aspects of six quinoa (*Chenopodium quinoa* Willd.) ecotypes from three geographical areas of Chile. Chilean J. Agric. Res. 72, 175–181.
Morison, J.I.L., Baker, N.R., Mullineaux, P.M., Davies, W.J., 2008. Improving water use in crop production. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363, 639–658.
O'Mara, F.P., 2012. The role of grasslands in food security and climate change. Ann. Bot. 110, 1263–1270.
Pérez-López, U., Robredo, A., Lacuesta, M., Mena-Petite, A., Muñoz-Rueda, A., 2009a. The impact of salt stress on the water status of barley plants is partially mitigated by elevated CO_2. Environ. Exp. Bot. 66, 463–470.
Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Muñoz-Rueda, A., Navari-Izzo, F., Mena-Petite, A., 2009b. The oxidative stress caused by salinity in two barley cultivars is mitigated by elevated CO_2. Physiol. Plant 135, 29–42.
Pérez-López, U., Robredo, A., Lacuesta, M., Sgherri, C., Mena-Petite, A., Navari-Izzo, F., Muñoz-Rueda, A., 2010. Lipoic acid and redox status in barley plants subjected to salinity and elevated CO_2. Physiol. Plant 139, 256–268.
Pérez-López, U., Miranda-Apodaca, J., Muñoz-Rueda, A., Mena-Petite, A., 2013. Lettuce production and antioxidant capacity are differentially modified by salt stress and light intensity under ambient and elevated CO_2. J. Plant Physiol. 170, 1517–1525.
Pérez-López, U., Mena-Petite, A., Muñoz-Rueda, A., 2014. Will carbon isotope discrimination be useful as a tool for analysing the functional response of barley plants to salinity under the future atmospheric CO_2 conditions? Plant Sci. 226, 71–81.
Panuccio, M.R., Jacobsen, S.E., Akhtar, S.S., Muscolo, A., 2014. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB Plants 6, plu047.
Pulvento, C., Riccardi, M., Lavini, A., Iafelice, G., Marconi, E., d'Andria, R., 2012. Yield and quality characteristics of quinoa grown in open field under different saline and non-saline irrigation regimes. J. Agron. Crop Sci. 198, 254–263.
R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Razzaghi, F., Ahmadi, S.H., Jacobsen, S.E., Jensen, C.R., Andersen, M.N., 2012a. Effects of salinity and soil–drying on radiation use efficiency, water productivity and yield of quinoa (*Chenopodium quinoa* Willd.). J. Agron. Crop Sci. 198, 173–184.
Razzaghi, F., Plauborg, F., Jacobsen, S.-E., Jensen, C.R., Andersen, M.N., 2012b. Effect of nitrogen and water availability of three soil types on yield: radiation use efficiency and evapotranspiration in field-grown quinoa. Agric. Water Manag. 109, 20–29.
Razzaghi, F., Jacobsen, S.-E., Jensen, C.R., Andersen, M.N., 2015. Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought – mechanisms of tolerance. Funct. Plant Biol. 42, 136–148.
Riccardi, M., Pulvento, C., Lavini, A., d'Andria, R., Jacobsen, S.E., 2014. Growth and ionic content of quinoa under saline irrigation. J. Agron. Crop Sci. 200, 246–260.
Ruiz, K.B., Biondi, S., Martínez, E.A., Orsini, F., Antognoni, F., Jacobsen, S.E., 2016. Quinoa – a model crop for understanding salt-tolerance mechanisms in halophytes. Plant Biosyst. 150, 357–371.
Ruiz-Carrasco, K., Antognoni, F., Coulibaly, A.K., Lizardi, S., Covarrubias, A., Martínez, E.A., Molina-Montenegro, M.A., Biondi, S., Zurita-Silva, A., 2011. Variation in salinity tolerance of four lowland genotypes of quinoa (*Chenopodium quinoa* Willd.) as assessed by growth physiological traits, and sodium transporter gene expression. Plant Physiol. Biochem. 49, 1333–1341.
Shabala, L., Mackay, A., Tian, Y., Jacobsen, S.-E., Zhou, D., Shabala, S., 2012. Oxidative stress protection and stomatal patterning as components of salinity tolerance mechanism in quinoa (*Chenopodium quinoa*). Physiol. Plant. 146, 26–38.
Silveira, J.A.G., Melo, A.R.B., Viégas, R.A., Oliveira, J.T.A., 2001. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. Environ. Exp. Bot. 46, 171–179.
Singh, S.K., Sharma, H.C., Goswami, A.M., Datta, S.P., Singh, S.P., 2000. In vitro growth and leaf composition of grapevine cultivars as affected by sodium chloride. Biol. Plant. 43, 283–286.
Smith, S.J., Thomson, A.M., Rosenberg, N.J., Izaurralde, R.C., Brown, R.A., Wigley, T.M.L., 2005. Climate change impacts for the conterminous USA: An integrated assessment. Part 1. Scenarios and context. In: Rosenberg, N., Edmonds, J. (Eds.), Climate Change Impacts for the Conterminous USA. Springer, Netherlands, pp. 7–25.
Sun, Y., Liu, F., Bendevis, M., Shabala, S., Jacobsen, S.E., 2014. Sensitivity of two quinoa (*Chenopodium quinoa* Willd.) varieties to progressive drought stress. J. Agron. Crop Sci. 200, 12–23.
Talebnejad, R., Sepaskhah, A.R., 2016. Physiological characteristics, gas exchange, and plant ion relations of quinoa to different saline groundwater depths and water salinity. Arch. Agron. Soil Sci. 62, 1347–1367.
Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. U. S. A. 108, 20260–20264.
United Nations, Department of Economics and Social Affairs, Population Division, 2015. World Population Prospects: The 2015 Revision, Key Findings and Advances Tables. Working Paper No. ESA/P/WP.241.
von Caemmerer, S., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387.
Wellburn, A.R., 1994. The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313.